Online-Only Abstracts

Effectiveness of bacteriophages in the sputum of cystic fibrosis patients

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Abstract

Bacteriophages have been shown to be effective for treating acute infections of the respiratory tract caused by antibiotic-resistant bacteria in animal models, but no evidence has yet been presented of their activity against pathogens in complex biological samples from chronically infected patients. We assessed the efficacy of a cocktail of ten bacteriophages infecting *Pseudomonas aeruginosa* following its addition to 58 sputum samples from cystic fibrosis (CF) patients collected at three different hospitals. Ten samples that did not contain *P. aeruginosa* were not analysed further. In the remaining 48 samples, the addition of bacteriophages led to a significant decrease in the levels of *P. aeruginosa* strains, as shown by comparison with controls, taking two variables (time and bacteriophages) into account ($p = 0.024$). In 45.8% of these samples, this decrease was accompanied by an increase in the number of bacteriophages. We also tested each of the ten bacteriophages individually against 20 colonies from each of these 48 samples and detected bacteriophage-susceptible bacteria in 64.6% of the samples. An analysis of the clinical data revealed no correlation between patient age, sex, duration of *P. aeruginosa* colonization, antibiotic treatment, FEV1 (forced expiratory volume in the first second) and the efficacy of bacteriophages. The demonstration that bacteriophages infect their bacterial hosts in the sputum environment, regardless of the clinical characteristics of the patients, represents a major step towards the development of bacteriophage therapy to treat chronic lung infections.

Clinical and virological characteristics associated with severe acute hepatitis B

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Abstract

To identify early predictors of a severe or fulminant course in patients with acute viral hepatitis B (AVH-B). One hundred and thirty-eight patients with symptomatic acute hepatitis B observed from 1999 to 2012 were enrolled. For each patient, the demographics, risk factors for the acquisition of hepatitis B virus (HBV) infection, clinical, biochemical and virological data (HBV DNA, HBV DNA sequences) were recorded and analysed. The HBV mutants in the polymerase region were sought in 110 (87%) patients by direct sequencing, and the rtM204V/I mutations also by an allele-specific PCR. AVH-B was severe in 13 (9.4%) of the 138 patients enrolled, fulminant in 6 (4.3%) and with a normal clinical course in 119. The 19 patients with severe or fulminant AVH-B more frequently than the 119 with a normal course stated intravenous drug use (63.2% versus 36.1%, p 0.04) and were HBV-DNA negative (31.6% versus 11.8%, p 0.03) and anti-hepatitis C virus (HCV) positive (57.9% versus 19.3%, p 0.0008); the prevalences of different HBV genotypes and of the rtM204V/I mutant were similar in these three forms of AVH-B. A multivariate logistic regression analysis identified a pre-existing HCV chronic infection as the only factor independently associated with a severe or fulminant clinical course of AVH-B (OR 4.89, 95% CI 1.5–15.94, p 0.01). A pre-existing HCV chronic infection was identified as the only factor independently associated with a severe clinical presentation of acute hepatitis B, an association most probably due to the combination of the liver lesions caused by acute hepatitis B and the pre-existing histological abnormalities related to HCV chronic infection.

Distinctive intrahepatic characteristics of paediatric and adult pathogenesis of chronic hepatitis C infection

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Abstract

Mechanisms leading to liver damage in chronic hepatitis C (CHC) are being discussed, but both the immune system and the virus are involved. The aim of this study was to evaluate intrahepatic viral infection, apoptosis and portal and periportal/interface infiltrate in paediatric and adult patients to elucidate the pathogenesis of chronic hepatitis C. HCV-infected, activated caspase-3^+ and $TUNEL^+$ hepatocytes, as well as total, $CD4^+$, $CD8^+$, $Foxp3^+$ and $CD20^+$ lymphocytes infiltrating portal and periportal/interface tracts were evaluated in 27 paediatric and 32 adult liver samples by immunohistochemistry or immunofluorescence. The number of infected hepatocytes was higher in paediatric than in adult samples (p 0.0078). In children, they correlated with apoptotic hepatocytes (activated caspase-$3^+ r = 0.74$, $p < 0.0001$; $TUNEL^+ r = 0.606$, p 0.0017). Also, infected ($p = 0.026$) and apoptotic hepatocytes ($p = 0.03$) were associated with the severity of fibrosis. In adults, activated caspase-3^+ cell count was increased in severe hepatitis ($p = 0.009$). Total, $CD4^+$,

$CD8^+$ and $Foxp3^+$ lymphocyte count was higher in adult samples ($p < 0.05$). Paediatric $CD8^+$ cells correlated with infected ($r = 0.495$, p 0.04) and $TUNEL^+$ hepatocytes ($r = 0.474$, $p = 0.047$), while adult ones correlated with activated caspase-3^+ hepatocytes ($r = 0.387$, p 0.04). In adults, $CD8^+$ was associated with hepatitis severity ($p < 0.0001$) and correlated with inflammatory activity ($CD8^+$ $r = 0.639$, p 0.0003). HCV, apoptosis and immune response proved to be involved in CHC pathogenesis of both paediatric and adult patients. However, liver injury in paediatric CHC would be largely associated with a viral cytopathic effect mediated by apoptosis, while in adults it would be mainly associated with an exacerbated immune response.

Detection of small amounts of human adenoviruses in stools: comparison of a new immuno real-time PCR assay with classical tools

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Abstract

The detection of low virus concentrations in biological matrices, especially stool samples, is facing significant limitations as far as common diagnostic methods (enzyme-linked-immunosorbent assay (ELISA) or quantitative real-time PCR (qPCR)) are considered. Here the development of a new immuno real-time PCR (iPCR) is described and its performance in the detection of human adenoviruses (HAdVs) in spiked stools is compared with those of ELISA and qPCR assays. For the iPCR, detection of the sandwich formed by the complexation of capture antibody-antigen-detection antibody was performed by qPCR thanks to the substitution of peroxydase by a chimeric DNA. This modification increased the detection sensitivity 200-fold compared to ELISA. The direct qPCR results revealed that only 0.3–9.5% of the spiked HAdV were detectable, resulting from important losses of DNA occurring at the extraction step. This step was not necessary in the iPCR workflow, avoiding this drawback. The losses of viral particles occurred at the elution step from the stool only. The recovery rate of the iPCR was thus better and ranged between 21 and 54%. As a result, iPCR enabled the detection of lower virus concentrations in stool samples compared to those detected by ELISA and qPCR. The iPCR could be considered as a 'hyper sensitive ELISA' for early detection of HAdV infections, especially in the case of immunocompromised patients after haematopoietic stem cell transplant.